Denitrification Enzyme Activity in Swine Wastewater Effluent of a Nitrification/Denitrification Treatment System

P. G. Hunt, T. A. Matheny, M. B. Vanotti, K. C. Stone, A. A. Szogi

Abstract. *Intensification of swine production in the U.S. and around the world requires advanced manure management. For swine manure management in the state of North Carolina, one system met all of the required advanced management criteria, and it was qualified as an environmentally superior technology. This investigation was part of the testing for this superior technology. The objectives of this investigation were to assess: (1) the denitrification enzyme activity (DEA) in the treatment system's homogenization tank and denitrification tank, and (2) the impact of the wastewater characteristics on this DEA. The DEA was measured by the acetylene inhibition method. Wastewater in the homogenization tank was fresh-flushed directly from the swine houses. Consequently, it was more concentrated than wastewaters in either the denitrification tank or typical swine wastewater lagoons; it had soluble biochemical oxygen demand (sBOD) of 676 mg L^{-1} and an electrical conductivity (EC) of 8.9 mS cm^{-1}. However, the DEA in the homogenization tank was significantly limited by the low level of NO_3-N, which was 0.1 mg L^{-1}. Conversely, the DEA of the denitrification tank was limited by its lower level of carbon; it had only 53 mg L^{-1} sBOD. However, it had a NO_3-N concentration of 150 mg L^{-1}. When non-limiting glucose-C and NO_3-N were added to the wastewaters of the homogenization and denitrification tanks, the homogenization tank had a significantly higher level of potential DEA: 17,943 vs. 10,055 mg N_2O-N m^{-3} d^{-1}, respectively. The DEA was generally well correlated by stepwise regression to the measured physiochemical characteristics. The findings of this investigation document that the DEA within this treated swine wastewater can be altered by manageable constituents of the processed swine wastewater, in particular soluble carbon and oxidized nitrogen.*

Keywords. *Carbon, Denitrification, Nitrate, Swine wastewater, Treatment system.*

With the intensification of swine production into fewer and larger farms in the U.S. and around the world, there has been much greater interest in superior waste management systems (Chastain et al., 1999; Key et al., 2009; Williams, 2001). This is particularly true in North Carolina, where there was rapid growth of swine production in the last decade of the 20th century (Barker, 1996a, 1996b). The waste management challenges of this growth caused the state to implement a moratorium on swine numbers and to develop an agreement with swine producers to seek environmentally superior technologies (EST) (Williams, 2002). The prevailing technology in North Carolina was and remains anaerobic lagoons with adjoining spray fields to receive the irrigated lagoon effluent (Bicudo et al., 1999; Harper et al., 2000). There were and continue to be many debated issues concerning the environmental and social aspects associated with these anaerobic lagoons (Aneja et al., 2000; Mallin, 2000; Schiffman et al., 2001; Stone et al., 1995; Stone et al., 1998; Williams, 2001, 2002). Accordingly, the emphasis was to find technologies that were superior to the anaerobic lagoon. One of the tested systems met all of the criteria and was qualified as an EST (Vanotti et al., 2007).

Whereas the flushed swine wastewater was high in both organic and inorganic nutrient loads, this system used a new approach. It separated the solids from the liquids through the use of polyacrylamide flocculation and mechanical separation (Vanotti and Hunt, 1999; Vanotti et al., 2002). It also used a high concentration of nitrifying bacteria (Vanotti and Hunt, 2000). The system removed over 90% of the nitrogen and phosphorus. When the treated wastewater was applied to bermudagrass via subsurface drip irrigation in a sandy soil, it was a very good source of supplemental nutrients and water for forage (Burns et al., 2009; Cantrell et al., 2009; Cantrell et al., 2010; Stone et al., 2008). One of the other aspects of the evaluation was to assess the denitrification enzyme activity (DEA) within the treatment system. This technique provided insight into the actual enzyme activity, the limitation of nitrate sources, the carbon sources, and the amount of incomplete denitrification relative to complete denitrification (Hunt et al., 2003). This technique has been used to evaluate denitrification in agronomic soils, riparian buffers, treatment wetlands, and anaerobic lagoons (Abbasi and Müller, 2011; Barton et al., 1998; Barton et al., 2000; Flite III et al., 2001; Gardner and White, 2010; Hunt et al., 2003, 2007, 2010; Liu et al., 2011; Miller et al., 2009).

Submitted for review in September 2011 as manuscript number SW 9355; approved for publication by the Soil & Water Division of ASABE in January 2012.

Mention of trade names, proprietary products, or vendors is for information only and does not constitute a guarantee or warranty of the product by the USDA and does not imply its approval to the exclusion of other products or vendors that may also be suitable. The USDA is an equal opportunity provider and employer.

The authors are **Patrick G. Hunt, ASABE Member,** Soil Scientist, **Terry A. Matheny,** Soil Scientist (Retired), **Matias B. Vanotti, ASABE Member,** Soil Scientist, **Kenneth C. Stone, ASABE Member,** Agricultural Engineer, and **Ariel A. Szogi, ASABE Member,** Soil Scientist, USDA-ARS Coastal Plains Soil, Water, and Plant Research Center, Florence, South Carolina. **Corresponding author:** Patrick G. Hunt, USDA-ARS Coastal Plains Soil, Water, and Plant Research Center, 2611 W. Lucas Street, Florence, SC 29501-1242; phone: 843-669-5203; e-mail: Patrick.hunt@ars.usda.gov.

The objectives of this investigation were to assess: (1) the DEA in both the homogenization tank for the flushed swine wastewater and the denitrification tank of the nitrification/denitrification treatment loop, and (2) the impact of the wastewater characteristics on the DEA.

Materials and Methods

A study on a full-scale system was conducted to evaluate DEA from various components of an innovative swine wastewater treatment system. The treatment system was initiated in 2003 at the Goshen Ridge Farm in Duplin County, North Carolina. A schematic of the treatment system is presented in figure 1. The treatment system and its wastewater treatment effectiveness are described in detail by Vanotti et al. (2007). It is briefly described in the following paragraph.

Manure was collected under the barns (4360 finishing pigs) using slatted floors and a pit-recharge system (Barker, 1996c). The pits were flushed each week. Wastewater was pumped from the swine houses to the treatment system, which combined solid-liquid separation with removal of nitrogen (N) and phosphorus (P) from the liquid phase (Vanotti et al., 2007). In the treatment system, the flushed manure was mixed (homogenization tank), flocculated using polyacrylamide, and the solids were separated from the liquid (Vanotti and Hunt, 1999; Vanotti et al., 2002). The separated liquid was pumped to a recycling nitrification/denitrification module, where: (1) in a nitrification tank, immobilized nitrifying bacteria (Vanotti and Hunt, 2000) transformed the ammoniacal N into nitrate-N, and (2) in a denitrification tank, denitrifying sludge transformed the nitrate-N into N_2 gas. This N removal module had a pre-anoxic modified Ludzack-Ettinger process configuration (Tchobanoglous et al., 2003) with internal recycling of nitrified liquid and settled activated sludge (RAS) into the denitrification tank at a rate of 4.4 and 1.8 times the inflow rate, respectively (Vanotti et al., 2007). The N treated liquid was then pumped to a P removal module, where alkaline treatment of the wastewater precipitated P as calcium phosphate and killed pathogens (Vanotti et al., 2003). One-third of the treated wastewater was recycled to the pit-recharge system, and the remainder was stored in a lagoon for subsequent land application. The system treated an average of 39 m^3 d^{-1} of raw manure flushed from the barns.

For this DEA study, 1 L of wastewater was manually collected from the homogenization and denitrification tanks on eight dates in 2003-2004. The samples were stored on ice, transported to the laboratory, and stored at 4°C until analysis. The DEA was measured by the acetylene inhibition method (Hunt et al., 2003; Tiedje, 1994). Wastewater samples (20 mL) from each sampling location were placed in 60 mL serum bottles (four bottles per sample per replication). All analyses were performed in triplicate. There were five DEA treatments (A through E). All treatments received 5 mL of chloramphenicol (1 g L^{-1}) to inhibit protein synthesis. Except for treatment E, all treatments also received 15×10^{-3} L of acetylene (produced from calcium carbide) to block denitrification at the nitrous oxide phase for measuring DEA. Treatments A through E were:

(A) Control: no glucose-C or nitrate-N added.
(B) Nitrate-N amended: 5 mL (200 mg L^{-1} NO_3-N).
(C) Glucose-C amended: 5 mL (600 mg L^{-1} glucose-C).
(D) Glucose-C and nitrate-N amended: 5 mL (600 mg L^{-1} glucose-C and 200 mg L^{-1} NO_3-N).
(E) Glucose-C and nitrate-N amended: 5 mL (600 mg L^{-1} glucose-C and 200 mg L^{-1} NO_3-N), no acetylene.

The serum bottles were capped with rubber septa, evacuated, and purged with purified nitrogen gas three times. Acetylene was added to the appropriate serum bottles after purging with nitrogen gas. The serum bottles were incubated on a horizontal shaker at 1.5 cycles s^{-1} and 24°C. After 1 and 5 h of incubation, 5×10^{-3} L of the headspace gases were removed from the serum bottles with a syringe (Plastipak, Franklin Lakes, N.J.) and injected into vials (borosilicate glass, crimp top with butyl septum). The N_2O-N in the headspace gas was measured with a gas chromatograph (model 3600 CX, Varian, Palo Alto, Cal.) equipped with a 15 mCi ^{63}Ni electron capture detector operating at 350°C. Chromatographic separation of the headspace gases was obtained by use of a 1.8 m long × 2 mm i.d. stainless steel column packed with Poropak Q (80-100 mesh, Alltech Associates, Deerfield, Ill.); the column and injector temperatures were 70°C, and the carrier gas was purified nitrogen. Samples were injected into the column with an autosampler (model 8200, Varian).

Wastewater analyses were performed according to APHA Standard Methods (Clesceri et al., 1998). Total suspended solids (TSS), volatile suspended solids (VSS), chemical oxygen demand (COD), and biochemical oxygen demand (BOD) were measured by Standard Methods 2540D, 2540E, 5220D, and 5210D, respectively. Ammonia-N (NH_3-N), total Kjeldahl N (TKN), nitrate-N (NO_3-N), orthophosphate (PO_4-P), and total phosphate (TP) were measured by Standard Methods 4500-NH_3 G, 4500-N_{org} D, 4500NO_3 F, 4500-PF, and 4500-PH, respectively. Alkalinity was determined by acid titration to the bromocresol green endpoint (pH = 4.5) and expressed as mg $CaCO_3$ L^{-1}. Redox potential and pH were measured with pH/temperature meters (Orion models 290A and 210A, respectively, Thermo Scientific, Beverly, Mass.).

All data analyses were conducted with SAS version 9.2 (SAS, 2002). For analysis of variance (ANOVA), the data were analyzed using the GLIMMIX procedure. For insight into the effects of wastewater characteristics upon denitrification, stepwise regression was used. For these regressions, the Cp values for the final step of the stepwise regressions did not exceed the value that corresponded to the number of variables used in the final regression step. Thus, these Mallow's Cp values were consistent with an acceptably low collinearity in the stepwise regression model.

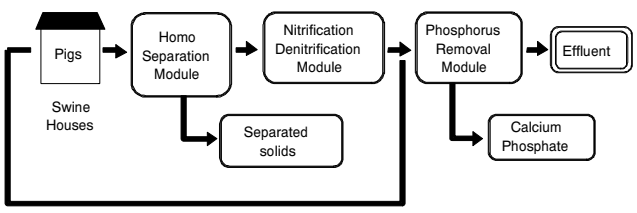

Figure 1. Schematic of the Goshen Ridge swine wastewater treatment system.

Results and Discussion

Wastewater Characteristics

The physicochemical characteristics of the flushed swine wastewater during the entire treatment system investigation were reported by Vanotti et al. (2007). During the dates of this DEA study, the physiochemical characteristics were similar to those of the entire investigation (table 1). To understand these data within the context of the overall swine treatment system, it is useful to compare them to data from typical anaerobic lagoons of the Carolinas reported by Hunt et al. (2010). In the Hunt et al. (2010) study, nine commercial anaerobic swine wastewater lagoons were assessed. The pH of these lagoons and the flushed water were quite similar: 7.7 and 7.4, respectively. While the EC of 8.9 mS cm^{-1} was somewhat higher than the EC 6.7 mS cm^{-1} found in lagoons, it was within the standard deviation of the Carolina lagoons. These higher concentrations were likely related to the recycling of the soluble phase of the wastewater within the system's treatment loop, as well as the fact that the flushed wastewater was fresh and the lagoon wastewaters had undergone bio-physiochemical degradation. The ortho-P of the flushed wastewater was 128 mg L^{-1}, which was about three times greater than the Carolina lagoon concentrations. The TSS and VSS means of 9724 and 6840 mg L^{-1}, respectively, for this flushed wastewater were about five to ten times greater than those for typical anaerobic swine lagoons (Bicudo et al., 1999; Hunt et al., 2010). The alkalinity of 4589 mg L^{-1} and the ammonia concentration of 704 mg NH_4-N L^{-1} were about double those reported for the lagoons. Likewise, the TKN of the flushed wastewater was more than three times higher than the reported lagoon concentrations. As with the lagoons, there was <0.2 mg L^{-1} nitrate-N or nitrite-N in the flushed swine wastewater. Moreover, prior to the treatment system, the physicochemical characteristics of the lagoons on this farm were similar to many of the Carolina lagoons (Vanotti and Szogi, 2008).

The wastewater characteristics of the denitrification tank were somewhat closer to the lagoon characteristics with the exception of the nitrate concentration, which was 150 mg L^{-1} (table 2). It should also be noted that the suspended components were dramatically lower than the flushed swine manure. This was because of both suspended solid separation prior to the denitrification tank and carbon consumption by microbial respiration within the denitrification tank. Most of the suspended solids in the denitrification tank came from the microbial denitrification sludge. The impacts of solid liquid separation and biological N treatment on wastewater components were presented by Vanotti et al (2007). In contrast to the suspended organic phase, the pH and ortho-P concentration in the denitrification tank were rather similar to that of the homogenization tank. The impact of the nitrification/denitrification loop of the treatment system can be seen in the ammonia concentration, which was much lower. It was reduced from 704 in the flushed wastewater to 149 mg NH_4-N L^{-1} in the denitrification tank. On the other hand, the NO_3-N circulated from the nitrification tank increased the nitrate concentration of the denitrification tank to 150 mg NO_3-N L^{-1}.

Table 1. Physicochemical characteristic of the homogenizing tank.[a]

Wastewater	Mean	SD	Max.	Min.
pH	7.5	0.2	7.9	7.4
Alkalinity	4589	1991	7722	1850
TS	10,578	5271	20,357	3218
TSS	9724	8319	27,800	1000
VSS	6840	6632	21,920	740
COD	14559	11020	33,860	2050
sCOD	2928	1845	5940	800
BOD	2481	1835	5820	287
sBOD	676	625	2040	50
TP	486	323	1039	148
Ortho-P	128	48	223	71
TKN-N	1328	735	2502	483
NH_4-N	704	275	1129	321
NO_2-N	0.1	0.3	1.0	0.0
NO_3-N	0.1	0.3	1.0	0.0
EC (mS cm^{-1})	8.90	3.47	16.28	4.42

[a] All values are in mg L-1 except EC; SD is standard deviation.

Table 2. Physicochemical characteristics of the wastewater in the denitrification tank.[a]

Wastewater	Mean	SD	Max.	Min.
pH	7.4	0.3	7.8	7.06
Alkalinity	1343	540	2153	550
TSS	3318	1613	6620	790
VSS	2597	1494	5660	680
sCOD	582	225	920	332
sBOD	53	101	318	4
Ortho-P	129	26	174	82
NH_4-N	149	62	249	83
NO_2-N	0.0	0.0	0.0	0.0
NO_3-N	150	122	410	19

[a] All values are in mg L^{-1}; SD is standard deviation.

Denitrification Enzyme Activity

In the homogenization tank, the DEA was 21 mg N_2O-N m^{-3} d^{-1} (table 3). This is about 25% of the 87 mg N_2O-N m^{-3} d^{-1} reported for anaerobic swine lagoons (Hunt et al., 2010). If the DEA of the homogenization tank is expressed in terms of DEA per gram of TSS, it would have been 1.4 μg N_2O-N g^{-1} sludge h^{-1}. This value is about 8% of the 18 μg N_2O-N g^{-1} sludge h^{-1} DEA found in the suspended layer of a treatment wetland that had received partially nitrified swine lagoon wastewater (Hunt et al., 2009). As in the wetland sludge layer, the denitrification in the homogenization tank was extremely nitrate limited. The nitrate-N limitation was related to the putative high denitrification in the pits of the house before the wastewater recycled to the homogenization tank (fig. 1). The nitrate-N limitation was dramatically demonstrated by the increase of DEA by 25-fold to 12,623 mg N_2O-N m^{-1} d^{-1} upon the addition of nitrate-N. This level of latent DEA in the homogenization tank demonstrated the significant capacity of the swine house pits to denitrify any nitrate entering the pits and to consequently augment the denitrification of the denitrification tank. Thus, the total treatment system loop had the microbial DEA capacity consistent with the removal of nitrate between the nitrification/denitrification loop and the homogenization tank (Vanotti et al., 2007). In contrast to the addition of nitrate, the addition of glucose-C did not significantly increase DEA. It was 434 mg N_2O-N m^{-3} d^{-1}. This clearly documents what would be reasonable to have assumed; i.e., wastewater flushed from the pits had high levels of DEA-supporting carbon. Yet when both nitrate-N and glucose-C

Table 3. DEA in the flushed wastewater homogenization tank.

Treatment	DEA (mg N_2O-N m^{-1} d^{-1})				
	LS Mean[a]	Mean[b]	SD	Max.	Min.
A	21 c	475	648	2113	0
B	12623 b	13039	14583	41723	134
C	434 c	651	841	3068	0
D	17943 a	17943	19536	60710	2254
E	1179 c	1179	1642	5587	0

[a] Means follow by the same letter are not significantly different at the 0.05 level by the LS means procedure.
[b] Arithmetic mean.

Table 4. DEA mean for the denitrification tank.

Treatment	DEA (mg N_2O-N m^{-1} d^{-1})			
	Mean[a]	SD	Max.	Min.
A	6288 b	5259	17542	864
B	6069 bc	4723	18074	601
C	8730 a	6274	24292	2736
D	10055 a	9613	35033	2752
E	3910 c	1899	7482	556

[a] Means follow by the same letter are not significantly different at the 0.05 level by the LS means procedure. The LS means and the arithmetic means were the same.

were added, the DEA was 17,943 mg N_2O-N m^{-3} d^{-1}. This increase in DEA documents the fact that, while high, the DEA-supporting carbon of the flushed wastewater is not limitless, although the carbon was sufficiently high to drive most of the denitrification to completion with N_2 as the end product. When no acetylene was added to block N_2 formation, the nitrous oxide produced with the addition of nitrate-N and glucose-C was 1179 mg N_2O-N m^{-3} d^{-1}. This was less than 7% of the total DEA of 17,943 mg N_2O-N m^{-3} d^{-1}.

In the denitrification tank, treatment A had a DEA of 6288 mg N_2O-N m^{-3} d^{-1} (table 4). This level of DEA in the denitrification tank is more than two orders of magnitude greater than the 21 mg N_2O-N m^{-3} d^{-1} measured for treatment A in the flushed wastewater homogenization tank. This is directly related to the presence of 145 mg nitrate-N L^{-1} in the denitrification tank; the DEA was not nitrate-N limited. This nitrate-N was a result of the proper operation of internal recycling of nitrified liquid into the denitrification tank. Accordingly, there was no increase in DEA with the addition of nitrate-N in treatment B. It was very similar to treatment A, with a DEA of 6069 mg N_2O-N m^{-3} d^{-1}. This lack of response to the nitrate-N in treatment B is in sharp contrast to the large DEA increase for treatment B in the homogenization tank, where nitrate was extremely limiting, with a mean of 0.1 mg L^{-1}. Similarly, this lack of response to nitrate-N was very different from that found in treatment wetlands and lagoons, where nitrate-N concentrations were almost always a DEA limiting factor (Hunt et al., 2003, 2006). However, when glucose-C was added to the denitrification tank wastewater; the DEA increased significantly to 8730 mg N_2O-N m^{-3} d^{-1}. Thus, there was considerable latent denitrification enzyme activity that was not being expressed in the denitrification tank because of carbon limitation. This fact is consistent with the reality that these enzymes moved through the system and completely denitrified the excess nitrate-N in the wastewater pits of the swine houses. In the function of the wastewater treatment system, the recycling wastewater entered these houses with excess nitrate-N of 224 mg L^{-1}, but it had less than 1 mg L^{-1} when it recycled back to the homogenization tank (Vanotti et al., 2007). When both nitrate-N and glucose-C were added to the denitrification tank wastewater in treatment D, the DEA was 10,055 mg N_2O-N m^{-3} d^{-1}. This DEA was significantly greater than the DEA of treatments A and B, where no carbon was added. Yet this level of DEA was not significantly greater than treatment C, where only carbon was added. Moreover, it was significantly smaller ($p \leq 0.05$) than treatment D in the homogenization tank, where carbon and nitrate were added to the wastewater.

These findings are consistent with the reasonable expectation that the homogenization tank would generally have sufficient non-limiting levels of carbon and that the denitrification tank would generally have non-limiting levels of nitrate. This difference in carbon/nitrogen balances suggests that the denitrification tank would likely have a higher percentage of incomplete denitrification than the 7% measured in the homogenization tank with treatment E. This was in fact the case for the denitrification tank; treatment E had 3910 mg N_2O-N m^{-3} d^{-1}. This represented 39% of the DEA in treatment D. The amount of nitrous oxide production from incomplete denitrification is known to be affected by the C/N ratio. The threshold ratio varies among systems and C/N constituents. It has been reported to be at ratios of 3 to 25 (Hunt et al., 2010; Hwang et al., 2006; Klemedtsson et al., 2005). In the case of wastewater treatment systems, Hwang et al. (2006) found that the threshold C/N ratio also varied with the type of denitrification system. In the denitrification tank, the ratio of soluble carbon to oxidized nitrogen was 11. These ratios were much lower than those found in the homogenization tank because it had high concentrations of soluble carbon and very low concentrations of oxidized nitrogen. Nonetheless, the major insight from treatment D in this investigation is not the exact percentage of nitrous oxide. It is, rather, documentation that the production of nitrous oxide from denitrification enzyme activity within this treated swine wastewater was altered by the amounts soluble of carbon and oxidized nitrogen.

Effect of Wastewater Characteristics on DEA

The effects of wastewater characteristics were assessed for each DEA treatment via stepwise regression. The evaluated physiochemical characteristics for the homogenization tank and the denitrification tank used in the stepwise regressions are presented in tables 1 and 2, respectively. For the control (treatment A) with wastewater from the homogenization tank, the DEA was well correlated to the VSS concentration. A simple linear regression provided an R^2 value of 0.73 (table 5). The regression equation was DEA = -9212 + 1205(VSS). If pH was added to the equation, the R^2 was increased slightly to 0.82. The VSS would represent carbon that would have both propelled the denitrification process in the presence of NO_3-N and would have initially been in wastewater pits of the houses. Thus, it is a characteristic that would be expected to be related to factors such as the manure load, the denitrification processes, and the DEA level in the flushed wastewater. In the case of treatment A in the denitrification tank wastewater, the best predictive characteristic was NH_4-N. When regressed along with the sCOD, the R^2 value was 0.78. This regression equation was DEA = -10,632 + 60.3(NH_4-N) + 13.6(sCOD).

Table 5. Stepwise regression of physiochemical variable affecting DEA for treatment A.

Variable	Slope	Partial R^2	Model R^2	C(p)	Pr > F
Homogenization tank[a]					
VSS	1205	0.73	0.73	9	<0.0001
pH	0.10	0.09	0.82	3	0.0121
Denitrification tank[b]					
NH_4	60.3	0.46	0.46	126	0.0008
sCOD	13.6	0.33	0.78	42	<0.0001

[a] Intercept for homogenization tank DEA = -9,212.
[b] Intercept for denitrification tank DEA = -10,632.

This regression is a realistic outcome, considering that the DEA is typically responsive to the nitrogen and carbon content of soils, sludges, and wastewaters (Hunt et al., 2009).

In the case of treatment B and the wastewater from the homogenization tank, the best correlation with DEA was TSS with an R^2 of 0.76 (table 6). If sBOD was added to the stepwise regression, the R^2 was increased to 0.92. The predictive equation was DEA = 45 + 2.5(TSS) – 16.9(sBOD). This was somewhat an expected carbon-driven equation, taking into account that treatment B had the addition of non-limiting NO_3-N. Therefore, its DEA would have likely been controlled by the available carbon parameters. In the case of treatment B in the denitrification tank, there was little increase in DEA upon addition of non-limiting NO_3-N. The best predictive physicochemical characteristic was oP, with an R^2 of 0.44. With the addition of the ratio of oP/(NH_4-N + NO_3-N) to the stepwise regression, the R^2 became 0.73. Finally, with the addition of sCOD, the R^2 was 0.85. The predictive equation was DEA = 1048 +37.4(oP) – 8700[oP/(NH_4-N + NO_3-N)] + 15.9(sCOD). In this treatment, the level of phosphorus became an important aspect of DEA. This is consistent with previous findings relative to the significance of P in denitrification treatment of wastewaters (Zeng et al., 2004).

In treatment C, in which non-limiting glucose-C was added to the wastewater of the homogenization tank, the best predictive parameter was TSS. The R^2 for the simple linear equation was 0.70 (table 7). The simple linear regression equation was DEA = -176 + 0.08(TSS). Whereas the DEA of treatment C did not differ significantly from treatment A, it would be expected to correlate with similar physiochemical parameters. However, in treatment A, the best correlation was with VSS rather than the more inclusive TSS. In either case, this parameter likely relates to the level of nitrate that came into the wastewater pits of the swine houses. In the denitrification tank, the DEA increased upon the addition of non-limiting glucose-C. Again, the DEA for treatment C was related to both the carbon and the oxidized nitrogen. The best

Table 6. Stepwise regression of physiochemical variable affecting DEA for treatment B.

Variable	Slope	Partial R^2	Model R^2	C(p)	Pr > F
Homogenization tank[a]					
TSS	2.5	0.76	0.76	261	<0.001
sBOD	-16.9	0.17	0.92	71	<0.001
Denitrification tank[b]					
oP	37.4	0.44	0.44	53	0.0010
oP/(NO_3 + NH_4)	-8700	0.29	0.73	19	0.0004
sCOD	15.9	0.12	0.85	6	0.0016

[a] Intercept for homogenization tank DEA = 45.
[b] Intercept for denitrification tank DEA = 1,048.

Table 7. Stepwise regression of physiochemical variable affecting DEA for treatment C.

Variable	Slope	Partial R^2	Model R^2	C(p)	Pr > F
Homogenization tank[a]					
TSS	0.08	0.70	0.70	3	<0.0001
Denitrification tank[b]					
VSS	4.6	0.55	0.55	77	0.0001
VSS/(NO_3 + NH_4)	-361	0.20	0.76	36	0.0011

[a] Intercept for homogenization tank DEA = -176.
[b] Intercept for denitrification tank DEA = 4,590.

fit parameter was VSS, with an R^2 of 0.55 (table 7). When the ratio of VSS/(NO_3-N + NH_4-N) was added to the stepwise regression, the R^2 improved to 0.76. The predictive equation was DEA = 4590 +4.6(VSS) – 361[VSS/(NO_3-N + NH_4-N)]. Thus, the level of DEA could be assessed by the amount of available carbon and the balance of this carbon to the oxidized nitrogen.

In treatment D with the homogenization tank wastewater to which both non-limiting glucose-C and nitrate-N were added, the DEA increased greatly. The best predictive parameter was TSS, with a simple linear regression R^2 of 0.56 (table 8). When sBOD and alkalinity were added to the stepwise regression, the R^2 improved to 0.97. The predictive equation was DEA = -11,083 + 3.1(TSS) – 40.9(sBOD) + 5.9(alkalinity). Thus, when the DEA potential was able to be exhibited in the presence of both non-limiting glucose-C and NO_3-N, there was an increase by a parameter that related to the total load of manure (TSS). There was a negative relationship to the sBOD; this seems reasonable because its consumption would be driven by denitrification. Additionally, the DEA increased with the alkalinity that was ostensibly from denitrification. In the case of the denitrification tank wastewater receiving treatment D, the best predicative parameter was oP. This is similar to treatment B, except the R^2 was slightly lower at 0.34. When VSS and pH were added to the stepwise regression, the R^2 improved to 0.78. The predictive equation was DEA = 12,5225 + 464(oP) + 5.4(VSS) – 25,898(pH). These again seem to be reasonable physicochemical parameters to be related to the DEA level of treatment D.

In treatment E, which had only chloramphenicol added, the best parameter for predicting N_2O-N levels for the homogenization tank was NH_4-N (table 9). It had a simple linear regression R^2 of 0.34. When sCOD and the ratio of sCOD/(NH_4-N + NO_3-N) were added to the stepwise regression, the R^2 increased to 0.78. The predictive equation was N_2O-N = -6636 + 14.5(NH_4-N) – 2.5(sCOD) + 1260[sCOD/(NH_4-N + NO_3-N)]. This finding is consistent

Table 8. Stepwise regression of physiochemical variable affecting DEA for treatment D.

Variable	Slope	Partial R^2	Model R^2	C(p)	Pr > F
Homogenization tank[a]					
TSS	3.1	0.56	0.56	666	<0.0001
sBOD	-40.9	0.33	0.89	154	<0.0001
Alkalinity	5.9	0.08	0.97	38	<0.0001
Denitrification tank[b]					
oP	464	0.34	0.34	48	0.0058
VSS	5.4	0.25	0.59	26	0.0040
pH	-25898	0.20	0.78	8	0.0011

[a] Intercept for homogenization tank DEA = -11,083.
[b] Intercept for denitrification tank DEA = 125,225.

Table 9. Stepwise regression of physiochemical variable affecting DEA for treatment E.

Variable	Slope	Partial R^2	Model R^2	C(p)	Pr > F
Homogenization tank[a]					
NH_4	14.49	0.34	0.34	1185	0.006
sCOD	-2.5	0.30	0.63	654	0.001
C/N[b]	1260	0.15	0.78	391	0.004
Denitrification tank[c]					
TSS	-0.68	0.1139	0.1139	22.8753	0.135
oP	73.0	0.1015	0.2154	20.3057	0.144
pH	6088	0.2002	0.4156	13.2977	0.027

[a] Intercept for homogenization tank DEA = -6,636.
[b] -sCOD/(NH_4-N + NO_3-N).
[c] Intercept for denitrification tank DEA = -30,931.

with previously published research (Hunt et al., 2007; Hwang et al., 2006; Klemedtsson et al., 2005). The N_2O-N level in treatment E for the denitrification tank was not well correlated to the regressed parameter. The best correlated parameter (TSS) provided an R^2 of only 0.11, and it was not significant at the 0.05 level. When oP and pH were added to the stepwise, the R^2 only improved to 0.42. It is possible that the C/N ratio of the treated wastewater along with the added glucose-C and NO_3-N was insufficiently high to control the level of incomplete denitrification. Under these conditions, unmeasured parameters were likely controlling the variation among the measured N_2O-N of treatment E in the denitrification tank wastewater.

CONCLUSION

The following conclusions can be drawn from this study:

- The DEA in the homogenization tank containing raw swine wastewater was significantly limited by the low level of NO_3-N, which was 0.1 mg L^{-1}.
- Conversely, the DEA of the denitrification tank in a biological N removal system after solid-liquid separation was limited by the lower level of carbon. There, the NO_3-N concentration was increased to 150 mg L^{-1}. However, the sBOD was lowered by the solid-liquid separation step. The denitrification had only 53 mg L^{-1} sBOD compared to the 676 mg L^{-1} of the homogenization tank.
- When non-limiting glucose-C and NO_3-N were added to the wastewaters of both the homogenization and denitrification tanks, the homogenization tank had a significantly higher level of DEA: 17,943 vs. 10,055 mg N_2O-N m^{-3} d^{-1}, respectively.
- The DEA for the control (treatment A) was well correlated by stepwise regression to the measured physiochemical characteristics. For the homogenization tank wastewater, the DEA was well correlated (R^2 of 0.73) to the VSS concentration. With the denitrification tank wastewater, a two-parameter stepwise regression provided an R^2 of 0.78 with NH_4-N and sCOD.
- When non-limiting NO_3-N was added to the wastewater from the homogenization tank, the stepwise regression had an R^2 of 0.92 with TSS and sBOD.
- When non-limiting glucose-C was added to the denitrification tank, the stepwise regression had an R^2 of 0.76 with the parameters VSS and VSS/(NO_3-N + NH_4-N).
- Where no acetylene was added (treatment E), the percentage of denitrification ending with N_2O-N production was higher for the denitrification tank than the homogenization tank. This was most likely related to the much higher levels of soluble carbon relative to nitrate-N in the homogenization tank.
- The major insight of this investigation is that the DEA within this treated swine wastewater can be altered by manageable constituents of the processed swine wastewater, in particular soluble carbon and oxidized nitrogen. Both of these constituents can be influenced by solids separation efficiency and internal recycling.

REFERENCES

Abbasi, M. K., and C. Müller. 2011. Trace gas fluxes of CO_2, CH_4, and N_2O in a permanent grassland soil exposed to elevated CO_2 in the Giessen FACE study. *Atmos. Chem. Phys. Discuss.* 11(2): 4199-4227.

Aneja, V. P., J. P. Chauhan, and J. T. Walker. 2000. Characterization of atmospheric ammonia emissions from swine waste storage and treatment lagoons. *J. Geophys. Res.-Atmos.* 105(D9): 11535-11545.

Barker, J. C. 1996a. Effects of manure management practices on air quality and animal performance in swine production buildings. Pub. No. EBAE 180-93. Raleigh, N.C.: North Carolina Cooperative Extension Service.

Barker, J. C. 1996b. Lagoon design and management for livestock waste treatment and storage. Pub. No. EBAE 103-83. Raleigh, N.C.: North Carolina Cooperative Extension Service.

Barker, J. C. 1996c. Swine production facility manure management: Pit recharge-lagoon treatment. Pub. No. EBAE 128-88. Raleigh, N.C.: North Carolina Cooperative Extension Service.

Barton, L., C. D. A. McLay, L. A. Schipper, and C. T. Smith. 1998. Procedures for characterising denitrification rates in a wastewater-irrigated forest soil. *Australian J. Soil Res.* 36(6): 997-1008.

Barton, L., L. A. Schipper, C. T. Smith, and C. D. A. McLay. 2000. Denitrification enzyme activity is limited by soil aeration in a wastewater-irrigated forest soil. *Biol. Fertil. Soils* 32(5): 385-389.

Bicudo, J. R., L. M. Safley Jr., and P. W. Westerman. 1999. Nutrient content and sludge volumes in single-cell recycle anaerobic swine lagoons in North Carolina. *Trans. ASAE* 42(4): 1087-1093.

Burns, J. C., K. C. Stone, P. G. Hunt, M. B. Vanotti, K. B. Cantrell, and D. S. Fisher. 2009. Intake and digestibility of 'coastal' bermudagrass hay from treated swine waste using subsurface drip irrigation. *J. Environ. Qual.* 38(4): 1749-1756.

Cantrell, K. B., K. C. Stone, P. G. Hunt, K. S. Ro, M. B. Vanotti, and J. C. Burns. 2009. Bioenergy from coastal bermudagrass receiving subsurface drip irrigation with advance-treated swine wastewater. *Bioresour. Tech.* 100(13): 3285-3292.

Cantrell, K. B., P. G. Hunt, K. S. Ro, K. C. Stone, M. B. Vanotti, and J. C. Burns. 2010. Thermogravimetric characterization of irrigated bermudagrass as a combustion feedstock. *Trans. ASABE* 53(2): 413-420.

Chastain, J. P., J. J. Camberato, J. E. Albrecht, and J. Adams. 1999. Swine manure production and nutrient content. Clemson, S.C.: Clemson University Cooperative Extension, Confined Animal Manure Managers Certification Program.

Clesceri, L. S., A. E. Greenberg, and A. D. Eaton. 1998. *Standard Methods for the Examination of Water and Wastewater.* 20th ed. Washington, D.C.: APHA, AWWA, WEF.

Flite III, O. P., R. D. Shannon, R. R. Schnabel, and R. R. Parizek. 2001. Nitrate removal in a riparian wetland of the Appalachian Valley and Ridge physiographic province. *J. Environ. Qual.* 30(1): 254-261.

Gardner, L. M., and J. R. White. 2010. Denitrification enzyme activity as an indicator of nitrate movement through a diversion wetland. *SSSA J.* 74(3): 1037-1047.

Harper, L. A., R. R. Sharpe, and T. B. Parkin. 2000. Gaseous nitrogen emissions from anaerobic swine lagoons: Ammonia, nitrous oxide, and dinitrogen gas. *J. Environ. Qual.* 29(4): 1356-1365.

Hunt, P. G., T. A. Matheny, and A. A. Szogi. 2003. Denitrification in constructed wetlands used for treatment of swine wastewater. *J. Environ. Qual.* 32(2): 727-735.

Hunt, P. G., M. E. Poach, T. A. Matheny, G. B. Reddy, and K. C. Stone. 2006. Denitrification in marsh-pond-marsh constructed wetlands treating swine wastewater at different loading rates. *SSSA J.* 70(2): 487-493.

Hunt, P. G., T. A. Matheny, and K. S. Ro. 2007. Nitrous oxide accumulation in soils from riparian buffers of a coastal plain watershed: Carbon/nitrogen ratio control. *J. Environ. Qual.* 36(5): 1368-1376.

Hunt, P. G., K. C. Stone, T. A. Matheny, M. E. Poach, M. B. Vanotti, and T. F. Ducey. 2009. Denitrification of nitrified and non-nitrified swine lagoon wastewater in the suspended sludge layer of treatment wetlands. *Ecol. Eng.* 35(10): 1514-1522.

Hunt, P. G., T. A. Matheny, K. S. Ro, M. B. Vanotti, and T. F. Ducey. 2010. Denitrification in anaerobic lagoons used to treat swine wastewater. *J. Environ. Qual.* 39(5): 1821-1828.

Hwang, S., K. Jang, H. Jang, J. Song, and W. Bae. 2006. Factors affecting nitrous oxide production: A comparison of biological nitrogen removal processes with partial and complete nitrification. *Biodegradation* 17(1): 19-29.

Key, N., W. D. McBride, and M. Ribaudo. 2009. Changes in manure management in the hog sector: 1998-2004. Economic Information Bulletin No. EIB-50. Washington, D.C.: USDA Economic Research Service.

Klemedtsson, L., K. Von Arnold, P. Weslien, and P. Gundersen. 2005. Soil CN ratio as a scalar parameter to predict nitrous oxide emissions. *Global Change Biol.* 11(7): 1142-1147.

Liu, W., G. Liu, and Q. Zhang. 2011. Influence of vegetation characteristics on soil denitrification in shoreline wetlands of the Danjiangkou Reservoir in China. *CLEAN* 39(2): 109-115.

Mallin, M. A. 2000. Impacts of industrial animal production on rivers and estuaries. *American Scientist* 88(1): 26-37.

Miller, M. N., B. J. Zebarth, C. E. Dandie, D. L. Burton, C. Goyer, and J. T. Trevors. 2009. Denitrifier community dynamics in soil aggregates under permanent grassland and arable cropping systems. *SSSA J.* 73(6): 1843-1851.

SAS. 2002. Statistical Analysis System. Version 9.2. Cary, N.C.: SAS Institute, Inc.

Schiffman, S. S., J. L. Bennett, and J. H. Raymer. 2001. Quantification of odors and odorants from swine operations in North Carolina. *Agric. Forest Meteorol.* 108(3): 213-240.

Stone, K. C., P. G. Hunt, S. W. Coffey, and T. A. Matheny. 1995. Water quality status of a USDA water quality demonstration project in the eastern coastal plain. *J. Soil Water Cons.* 50(5): 567-571.

Stone, K. C., P. G. Hunt, F. J. Humenik, and M. H. Johnson. 1998. Impact of swine waste application on ground and stream water quality in an eastern coastal plain watershed. *Trans. ASAE* 41(6): 1665-1670.

Stone, K. C., P. G. Hunt, J. A. Millen, M. H. Johnson, T. A. Matheny, M. B. Vanotti, and J. C. Burns. 2008. Forage subsurface drip irrigation using treated swine wastewater. *Trans. ASABE* 51(2): 433-440.

Tchobanoglous, G., F. L. Burton, and H. D. Stensel. 2003. *Wastewater Engineering: Treatment and Reuse*. 4th ed. Boston, Mass.: McGraw Hill.

Tiedje, J. M. 1994. Denitrifier enzyme activity (DEA). In *Methods of Soil Analysis, Part 2*: 256-257. 2nd ed. S. H. Mickelson and J. M. Bigham, eds. Madison, Wisc.: SSSA

Vanotti, M. B., and P. G. Hunt. 1999. Solids and nutrient removal from flushed swine manure using polyacrylamides. *Trans. ASAE* 42(6): 1833-1840.

Vanotti, M. B., and P. G. Hunt. 2000. Nitrification treatment of swine wastewater with acclimated nitrifying sludge immobilized in polymer pellets. *Trans. ASAE* 43(2): 405-413.

Vanotti, M. B., and A. A. Szogi. 2008. Water quality improvements of wastewater from confined animal feeding operations after advanced treatment. *J. Environ. Qual.* 37(supp. 5): S86-S96.

Vanotti, M. B., D. M. C. Rashash, and P. G. Hunt. 2002. Solid-liquid separation of flushed swine manure with PAM: Effect of wastewater strength. *Trans. ASAE* 45(6): 1959-1969.

Vanotti, M. B., A. A. Szogi, and P. G. Hunt. 2003. Extraction of soluble phosphorus from swine wastewater. *Trans. ASAE* 46(6): 1665-1674.

Vanotti, M. B., A. A. Szogi, P. G. Hunt, P. D. Millner, and F. J. Humenik. 2007. Development of environmentally superior treatment system to replace anaerobic swine lagoons in the USA. *Bioresour. Tech.* 98(17): 3184-3194.

Williams, C. M. 2001. Technologies to address air quality issues impacting animal agriculture. *Water Sci. Tech.* 44(9): 233-236.

Williams, C. M. 2002. CAFOs: Issues and development of new waste treatment technology. *Penn State Environ. Law Rev.* 10(2): 217-247.

Zeng, R. J., Z. Yuan, and J. Keller. 2004. Improved understanding of the interactions and complexities of biological nitrogen and phosphorus removal processes. *Rev. Environ. Sci. Biotech.* 3(3): 265-272.